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Phytochemical study and characterization of the fixed oil, extracted from the fruits of *Gardenia ternifolia*

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Abstract

This present study concerns the phytochemical study and the characterization of the fixed oil of the fruits of *Gardenia ternifolia* which is a plant recognized in several African pharmacopoeias for its therapeutic virtues. Its fruits, leaves and roots are used for the treatment of several infections. Thus, the overall objective of this study is to contribute to the valorization of *Gardenia ternifolia* fruits in phytomedicine and agri-food. Phytochemical screening is carried out using standard methods, based on coloring and precipitation reactions. The extraction of the fixed oil was done using a Soxhlet, providing an estimated yield of 3%. The evaluation of the chemical parameters of this oil according to the AFNOR standards (French Association for Standardization) made it possible to obtain the following results: iodine index (II) = 5.6 ± 0.07 gI /100 gcg; acid value (IA) = 137.2 ± 0.001 $mgKOH/gcg$; saponification index (IS) = 201.6 ± 0.04 $mgKOH/gcg$; peroxide index (IP) = 8.4 ± 0.03 $\mu gO2/gcg$ and ester index (IE) = 64.4 ± 0.039 $mgKOH/gcg$. Ultimately, with much higher extraction quantities, the fruit oil of *Gardenia ternifolia* can present an interesting and valuable pharmaco-nutritional potential in the food industry and in pharmacology.

Keywords: *Gardenia ternifolia*; Pharmacopoeias; Fixed oil; Phytochemistry

1. Introduction

Today, herbal treatments have become more and more essential due to the increasingly noted chemoresistance to modern drugs. As a result, scientists are constantly exploring new avenues, especially in the field of phytochemistry, with a view to finding new and more effective remedies to improve human health. However, we must not ignore the other enormous potential offered by flora, especially that of Africa. Indeed, the latter is full of many plant species used by people for a long time as sources of food, timber, energy and remedies against various somatic or spiritual ailments. In the tropics, there are many sources of vegetable oils available, but not exploited or not used optimally. *Gardenia ternifolia*, native to tropical and subtropical regions of South Asia, Australia and Oceania could be one such species. It is a plant very well known to the populations thanks to its richness in curative virtues. But, like any legume, the seeds of *Gardenia ternifolia* would also contain fat, just as usable in food or for other purposes.

This study is part of the aim of promoting the medicinal plants of the Senegalese flora in order to identify new alternative sources of vegetable oils for therapeutic purposes, agrifood, cosmetics and bioenergetics. The work of Farah and *al.* (2018) and Nureye and *al.* (2018) respectively indicated safety of the fruits and roots of the plant [**1**].

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Previous phytochemical studies carried out on the fruits of *Gardenia ternifolia* revealed the presence of many phenolic compounds such as: gallic tannins, catechin tannins, flavonoids and anthocyanins in the aqueous extract of the fruits [**2**].

2. Material and methods

2.1. Plant material used

The plant was harvested in December 2021 in the commune of Taïba Ndiaye, a town located in the region of Thiès, in the west of Senegal, with geographical coordinates 15°3'0" N and 16°52'60" W.

The plant material consisted of fruits of *Gardenia ternifolia*.

After harvest, the fruits were dried in the dark at room temperature and identified at the Institut Fondamental d'Afrique Noire (IFAN). They were ground using an electric grinder to finally obtain a fine powder and then stored in jars to avoid any contamination (**Figure 1**).

Fruit Fruit of powder

Figure 1 Image of fruit and fruit powder of *Gardenia ternifolia*

2.2. Extraction method

Extraction is a process used to selectively extract one or more compounds from a mixture, based on physical or chemical properties. The method used in this present work is Soxhlet extraction. The solvent is brought to a boil, condensed in the condenser and then falls back into the siphon tank containing the solid to be extracted in a cartridge of thick paper. The contact between the solvent and the product to be extracted lasts during the accumulation of solvent in the tank, then when the solvent reaches a certain level, it primes the siphon and returns to the flask carrying the dissolved substance. This cycle can be repeated several times, depending on how easily the product diffuses into the solvent. This extraction process makes it possible to reduce the use of large quantities of solvent.

2.3. Methods for characterizing fixed oils

A mass of 125 g of vegetable matter are rolled up in a filter paper serving as a cartridge and then placed in the extractor. 500 mL of solvent (hexane) is poured into a flask then the whole is refluxed for two (2 hours) to extract as much oil as possible.

At the end of the extraction, 10 cycles are obtained. The first cycle lasted 30 min compared to the others where the duration varies between five (5) and six (6) min. This is explained by the fact that for the first extraction, the solid must be completely wet and dissolve in the solvent before siphoning.

After evaporation of the solvent (hexane) by a rotary evaporator, the oil obtained is then subjected to various tests.

2.4. Characterization of the oil

2.4.1. Determination of indices

The determination of chemical indices is a simple and summary means of having a first analytical approach to the nature of a fatty substance.

2.4.2. Iodine number

The iodine index is defined as the number of grams of iodine fixed per 100 g of fat. It was determined using the method of Wijs' [**3**], [**4**]. According to the experimental protocol used, an excess of iodine chloride, called Wijs' reagent, is added to the fatty substance in solution in chloroform. After a few minutes of reaction, potassium iodide and distilled water are added. The iodine released is titrated with a titrated sodium thiosulphate solution (0.1 N) in the presence of starch paste.

The iodine value is calculated according to the formula:

$$I_I = \frac{(V_B - V_E) \times C_{Na_2S_2O_3} \times M_I \times 100 \times 10^{-3}}{m_{cg}}$$

2.4.3. Acid number

The acid number (I_A) corresponds to the content of free fatty acids contained in the oil. This characteristic accounts for the state of degradation of an oil insofar as the free fatty acids are products of degradation and more particularly of hydrolysis of triglycerides, the main constituents of the oil. This is the quantity of KOH in milligrams needed to neutralize the acidity contained in 1 g of fat. This index was determined according to the AFNOR NF T 60-204 standard [**5**].

The acid number is given by: $\boldsymbol{I_A = \frac{(V_E - V_B) \times C_{KOH} \times M_{KOH}}{m_{cg}}}$

V_B : Volume of KOH poured to neutralize the blank;
V_E: volume of KOH poured to neutralize the sample;
C_{KOH}: concentration of the KOH solution;
M_{KOH}: molar mass of KOH
m_{cg}: mass of fat used.

2.4.4. Saponification index

The saponification index ($IS_)$ is the quantity of potassium hydroxide, expressed in milligrams, necessary to saponify 1 g of fat. This is a saponification reaction of the triglycerides of the oil. This index is determined according to standard NF T 60-206 [**5**].

The saponification index is determined according to the following relationship:

$$I_s = \frac{(V_B - V_E) \times C_{HCl} \times M_{KOH}}{m_{cg}}$$

V_B: Volume of HCl poured to neutralize the blank
V_E: Volume of HCl poured to neutralize the sample
C_{HCl}: Concentration of the HCl solution
M_{KOH}: Molar mass of KOH
m_{cg}: Mass of fat used

2.4.5. Peroxide value

By peroxide index of a fatty substance is meant the number of micrograms of active oxygen contained in one gram of product capable of oxidizing potassium iodide with the release of iodine. Its determination made according to the AFNOR NF T 60-220 standard reflects the state of oxidation of the oil [5]. This index makes the possible to predict a subsequent deterioration of the organoleptic qualities of the oil but does not provide any information on the oxidative past of the oil.

The peroxide index is given by:

$$I_p = \frac{1000 \times (V_E - V_B)}{m_{cg}}$$

V_E: volume of thiosulphate poured to neutralize the sample
V_B: volume of thiosulphate poured to neutralize the white
m_{cg}: body fat mass

2.4.6. Ester index

The ester index (I_E) of a fatty substance is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the acids released by the hydrolysis of the esters contained in 1g of fatty substance. In particular, the ester index is equal to the saponification index for pure glycerides. In practice, this index is not measured experimentally, but rather is deduced by making the difference between the saponification index (I_S) and the acid index (I_A), i.e.:

$$I_E = I_S - I_A$$

3. Results and discussion

3.1. Extraction rate

A mass of 5 g of plant material were macerated with 2x25 mL of solvent, then the filtrate obtained was evaporated to dryness and then weighed. The results of this extraction are recorded in the table below.

Table 1 Extraction rates in different solvents

Extract	Mass obtained (g)	Mining rate (Mr) (%)
Hexanic	0.006	0.12
Ethyl acetate	0.012	0.24
Methanolic	0.064	1.28
Aqueous	0.593	11.86

The extraction rate is evaluated by:

$$\text{Mr} = \frac{\textbf{mass obtained}}{\textbf{mass of plant matter}} \times \mathbf{100}$$

The results obtained (**Table 1**) show that the extraction rate increases with the polarity of the solvent. This suggests the richness in secondary metabolites of *Gardenia ternifolia*. The lowest levels are obtained with hexane, which is the least polar solvent. And the highest rate was obtained with the most polar solvent, which is water.

3.2. Phytochemical screening results

Phytochemical screening of *Gardenia ternifolia* fruit extracts was carried out using the methods mentioned above. The following table presents the results obtained.

Table 2 Phytochemical screening results

Secondary metabolites	Hexane	Ethyl acetate (AcOEt)	Methanol (MeOH)	Aqueous
Polyphenols	-	+	++	++
Flavonoïds	-	-	+	-
Alkaloïds	+	-	+	-
Sterols et Polyterpenes	++	-	+	-

Leucoanthocyanins et Catechols	-	-	+	+
Coumarins	+	-	-	-
Saponins	-	-	-	-
Mucilage	-	-	-	-
Catechic tannins	+	-	+	-
Gallic tannins	-	-	-	++

+ = Important; **-** = Missing; **++** = Very important

The phytochemical tests carried out on the extracts of the fruits of *Gardenia ternifolia* revealed the presence of:

- Polyphenols in all extracts except the hexanic one
- Flavonoids only in the methanolic extract
- Alkaloids in methanolic extracts
- Sterols and polyterpenes in hexane and methanol extracts
- Leucoanthocyanins and catechols in methanolic and aqueous extracts
- Coumarins only in hexane
- Catechic tannins in hexanic and methanolic extracts
- Gallic tannins only in the aqueous extract

On the other hand, saponosides and mucilages were not identified in any extract.

Gardenia ternifolia is used in traditional Ethiopian and Sudanese medicine for the treatment of malaria. Nureye and *al.* (2018) evaluated in vivo the antimalarial potential of the methanolic extract of the roots of *Gardenia ternifolia*. The results of this study showed that this extract has curative and prophylactic effects up to 36 % to 63 %. In addition, it caused weight loss, a reduction in temperature and then prevented anemia. In the light of these results, they concluded that the root of *Gardenia ternifolia* has antimalarial potential. This same activity has been proven in Senegal by Diouf and *al.* (2005). According to these authors, the antimalarial effect is linked to the flavonoids present in the roots of the plant. By correlating our results with those obtained in the literature, we deduce that the methanolic extract of the fruits of *Gardenia ternifolia* also has an antimalarial potential linked to the flavonoids present in the fruits. This therefore gives the plant its antimalarial activity. The antioxidant, antihypertensive and anticancer activities of the plant can be justified by the strong presence of polyphenols in the fruits. Indeed, the antioxidant activity of polyphenols is recognized and could explain their potential role in the prevention of several diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases [**6**].

3.3. Results of fixed oil extraction

The oil content of plant material is estimated by:

$$T_h = \frac{m_h}{m_0} \times 100$$

m_{oil}: mass of oil extracted; m_{oil} = 4.07 g
m_0: mass of seed powder used; m_0= 12.05 g
Th= 3 %

This result indicates that the oil yield of the fruits of *Gardenia ternifolia* is quite interesting.
The oil has a dark green color. This shows that it contains pigments which would be at the origin of this coloring.
The chemical characterization of the oil extracted from the fruits of *Gardenia ternifolia* gave the following results:

These results (**Table 3**) reveal that the oil contained in the fruits of *Gardenia ternifolia* has a high acid index, which testifies well to the instability of the fatty acid esters which compose it but also the degradation of the oil. This is likely due to degradation resulting from the sample storage conditions and/or the relatively high temperature during extraction.

Table 3 Indices characterizing *Gardenia ternifolia* oil

Clues	Error	Content
Iodine values	0.07	$5.6 \pm 0.07\ g_I/100g_{cg}$
Acid numbers	0.001	$137.2 \pm 0.001\ mg_{KOH}/g_{cg}$
Saponification indices	0.04	$201.6 \pm 0.04\ mg_{KOH}/g_{cg}$
Peroxide indices	0.03	$8.4 \pm 0.03\ \mu g_{O_2}/g_{cg}$
Ester indices	0.039	$64.4 \pm 0.039\ mg_{KOH}/g_{cg}$

In the analysis of fats, it is the iodine number which represents the most useful constant, because it is in relation to the values of this index that the important division of vegetable oils into drying oils, semi-drying oils is based and not driers. Indeed, the iodine index tells us about the degree of establishment of the fatty acids contained in a given oil. It is directly related to the degree of oxidation of an oil. Thus, the more an oil is unsaturated (the more there are multiple C=C bonds), we can base ourselves on this quantity to evaluate the ease of the oil to go rancid, given that the more it contains establishments, the more it will be sensitive to oxygen. However, the value of the iodine number of this oil, 5.6 ± 0.07 g.I_2/100 g_{cg} of oil, makes it possible to classify this oil among the non-drying oils, whose iodine numbers are between 0 at 110 g.I2/100 g_{cg} of oil. The value of the iodine index found in this study is much lower than the iodine indexes of olive, groundnut and castor oils varying between 75 and 94 g.I_2/100 g of oil **[7]**. Based on this relatively low iodine value of *Gardenia ternifolia* oil, the conservation of this oil could be done without too much risk of auto-oxidation. This oil would be weakly concentrated in unsaturated fatty acids.

The acid index of a fatty substance is a good indicator to determine its deterioration. *Gardenia ternifolia* fruit oil has an acid number of 137.2 ± 0.001mg_{KOH}/g_{cg}. This acidity is much higher than the maximum value recommended for an edible oil. Knowing that a low acidity value characterizes the purity and stability of an oil at room temperature [**8**], this criterion not being satisfied for *Gardenia ternifolia* oil, it is therefore necessary that precautions be taken in order to limit a probable subsequent denaturation of this oil [**9**].

Knowledge of the saponification index of a fatty substance tells us about the length of the carbon chain of the acids constituting this fatty substance. The saponification index of a fatty substance is higher the shorter the fatty acid carbon chain. The saponification index of *Gardenia ternifolia* oil (201.6 ± 0.04 mg_{KOH}/g_{cg}) is close to that of cottonseed oil (189 - 198), sunflower oil (188 - 194), palm oil (190 - 209) and palm olein (194-202) [**10**] usually used in food.

The peroxide index is a very useful criterion for assessing the first stages of oxidative deterioration of an oil. The value of the peroxide value found in this study (8.4 ± 0.03 $\mu g_{O2}/g_{cg}$) is lower than that of edible oils; which indicates a non-advanced state of oxidation of the oil.

The value of ester index of *Gardenia ternifolia* oil, which is 64.4 ± 0.039 mg_{KOH}/g_{cg} of oil, is lower than its saponification index 201.6 ± 0.04 mg_{KOH}/g_{cg}. This means that this oil contains a significant amount of free fatty acids.

3.4. Determination of the average molecular weight of the main components of the oil

By exploiting the expression of the saponification index and the saponification reaction, we obtain:

$$\boldsymbol{M_{TG} = \frac{3 \times M_{KOH}}{I_s}}$$

And after calculation, the average molecular mass of the triglyceride is M_{TG}=833 g/mol, leading to an average molar mass of the fatty acid equal to M_R=220 g/mol.

After comparison with the results of the literature, this fatty acid could well be palmitic acid of formula $C_{15}H_{31}COOH$ or $CH_3(CH_2)_{14}COOH$.

4. Conclusion

In this work, the phytochemistry of the fruits of *Gardenia ternifolia* was studied and the chemical characteristics of its oil were determined. The phytochemical study revealed the presence of several chemical compounds in the fruits of this plant which would be responsible for the biological activities of the plant. The characteristics evaluated show that this oil can present interesting chemical properties overall. However, the oil yield and the acid index show that precautions must be taken to limit a deterioration in the chemical and functional quality of this oil. The overall analysis of the different properties studied makes it possible to say that this oil could be used in the food industry and/or in phytomedicine. Indeed, additional studies must be undertaken to assess its chemical composition in fatty acids and its toxicity before considering its use in food. In addition, the determination of other parameters such as the contents of mineral elements, chlorophyll pigments and total carotenoids of the seed oil of *Gardenia ternifolia* would inform us about its pharmaco-nutritional qualities, but also about the degree of acid unsaturation of fats contained in the oil thanks to the determination of the iodine index.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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